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Ring Chromosome X in a Child With Manifestations of Kabuki Syndrome

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A female patient with the karyotype 45, X/46, X, r(X)(p11.2 q13) and severe developmental delay, prominent fingertip pads, long palpebral fissures, short stature, and history of hypotonia had a phenotype reminiscent of Kabuki syndrome. We hypothesized that overexpression of X chromosome-derived sequences might be associated with the Kabuki-like phenotype observed. The nature and parental origin of this small-ring X were ascertained using a combination of genotyping with microsatellite markers and quantitative Southern blotting. PCR-based genotyping demonstrated heterozygosity at X-linked loci SBMA (Xq11–q12) and DXS227 (Xq13.1). Hemizygosity was observed at several loci: DMD STR-49 (Xp21.2), DXS1003 (Xp11.23), DXS988 (Xp11.21), DXS101 (Xq21.3), FMR-1 (Xq27.3), and DXYS64 (Xq28). This ring X chromosome is paternally derived since only maternal alleles are inherited at three informative microsatellite loci. Results of FISH and RT-PCR experiments indicate that the XIST locus is missing in the ring X chromosome and not expressed. These data indicated a large deletion of the X chromosome consistent with a small-ring X chromosome with approximate breakpoints near p11.2 and q13. These results are comparable to the observation of others where an atypically severe phenotype has been associated with the presence of an r(X), or small mar(X). Am. J. Med. Genet. 70:37–42, 1997. © 1997 Wiley-Liss, Inc.

KEY WORDS: ring chromosome; Kabuki syndrome; XIST; X chromosome

INTRODUCTION

Although patients with a small ring or marker X chromosome usually have short stature and premature ovarian failure, most have a phenotype much more severe, specifically with respect to cognitive deficits than individuals with Ullrich-Turner syndrome (UTS) on the basis of other chromosome abnormalities. This unexpected phenotype was hypothesized to result from the loss of the X inactivation center at Xq13.2, resulting in functional disomy for genes in the pericentromeric region of the X [Van Dyke et al., 1992; Grompe et al., 1992; Migeon et al., 1993, 1994; Wolff et al., 1994; Jani et al., 1995]. Although no distinct pattern of malformations was seen in all patients with a small-ring X, several individuals were noted to have findings reminiscent of the Kabuki syndrome.

Kabuki syndrome is a recognizable pattern of malformation of unknown cause characterized by: mental retardation, postnatal growth deficiency, long-appearing palpebral fissures, lower palpebral fissure eversion, prominent pinnae, preauricular pits, prominent finger pads, and skeletal anomalies. Males and females are equally affected [Niikawa et al., 1988]. Most cases are sporadic; however, vertical transmission was reported twice [Halal et al., 1989; Kobayashi and Sakuragawa 1996].

Although most individuals with Kabuki syndrome have normal karyotypes, several affected individuals with a chromosome abnormality have been reported. Niikawa et al. [1988] described two of 62 affected children with a ring X or ring Y chromosome. A male patient with an inversion of the Y was also reported. However, his phenotypically normal father carried the same inversion, suggesting the inversion was not associated with Kabuki syndrome in this affected boy. Dennis et al. [1993] reported on three patients with a ring X chromosome and manifestations suggestive of Kabuki syndrome. Wellesley and Slaney [1994] reported on a 2-year-old girl with 45, X and anomalies of UTS and Kabuki syndrome. Additional patients with Kabuki syndrome traits have also been associated with other chromosome abnormalities, including an unbalanced translocation of 6q and 12q [Jardine et al., 1993], inversion of 4p [Fryns et al., 1994], a pseudodicentric

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chromosome 13 [Lynch et al., 1995], and an apparently balanced translocation of 15q and 17q [Glan-Gomez et al., 1995]. The relationship between documented chromosome abnormalities and the Kabuki phenotype is unclear.

We report on the molecular and cytogenetic characterization of a female patient with a small-ring X chromosome and several clinical signs suggestive of Kabuki syndrome. These data are consistent with the hypothesis that functional disomy for pericentromeric sequences on the X chromosome may be responsible for the abnormal phenotype seen in females with a small-ring or marker X chromosome. We speculate that overexpression of specific sequences, present in some individuals but not in others, could account for the Kabuki-like phenotype that occurs in some patients.

CLINICAL REPORT AND METHODS

Clinical Report

The patient is a Vietnamese girl originally evaluated at age 5 months for linear growth deficiency and profound hypotonia. At 5 months her length (60 cm) and weight (5.2 kg) were at the third centile for age. Her head circumference (OFC) (42 cm) was at the 50th centile. She had poor head and active muscle control. Epicanthal folds were noted along with loose skin over hands and feet. Her dermatoglyphic patterns included 9 tall ulnar loops. Laboratory evaluation included chromosome analysis (at another laboratory), thyroid function studies, CPK levels, EMG and nerve conduction studies, and a muscle biopsy. All test results were normal except for a karyotype of 45, X.

Despite her atypical presentation, she was given the diagnosis of UTS. Endocrine studies included FSH and LH levels that were consistent with gonadal dysgenesis. A pelvic ultrasound study showed a normal uterus, but no ovaries. Delays in physical growth and cognitive and speech development remained atypically severe for UTS. Her height remained two standard deviations below the mean for age using an UTS growth chart. Bone age was delayed and somatomedin levels were low. Growth hormone therapy was initiated at age 6 7/12 and by the age 9 5/12 her height was at the 50th centile for UTS girls. Developmental testing showed severe delays in cognitive functioning with marked deficits in communication. She was also diagnosed with attention deficit and hyperactivity.

She developed hirsutism and premature adrenarche at age 7 with normal testosterone and DHEA levels. She was noted to have recurrent urinary tract infections and recurrent episodes of otitis media (the latter necessitating the placement of myringotomy tubes). During a follow-up evaluation in the genetics clinic at 9 5/12 years, her atypical UTS was reviewed and a chromosome analysis was repeated in our laboratory. She had long palpebral fissures (2.5 cm) with prominent lower lashes and thick eyebrows (Fig. 1), a high arched palate, and flatter facial configuration than her parents. Her hands also showed prominent fingertip pads and a short fourth finger. These physical signs, along with the developmental delay, short stature, premature adrenarche, recurrent infections, and history of hypotonia suggested the diagnosis of Kabuki syndrome. In fact, this patient had the 5 main clinical manifestations of Kabuki syndrome [Niikawa et al., 1988]: peculiar face (100%); skeletal anomaly (92%); dermatoglyphic abnormality (93%); IQ < 80 (92%); and short stature (<–2.0 SD)(73%).

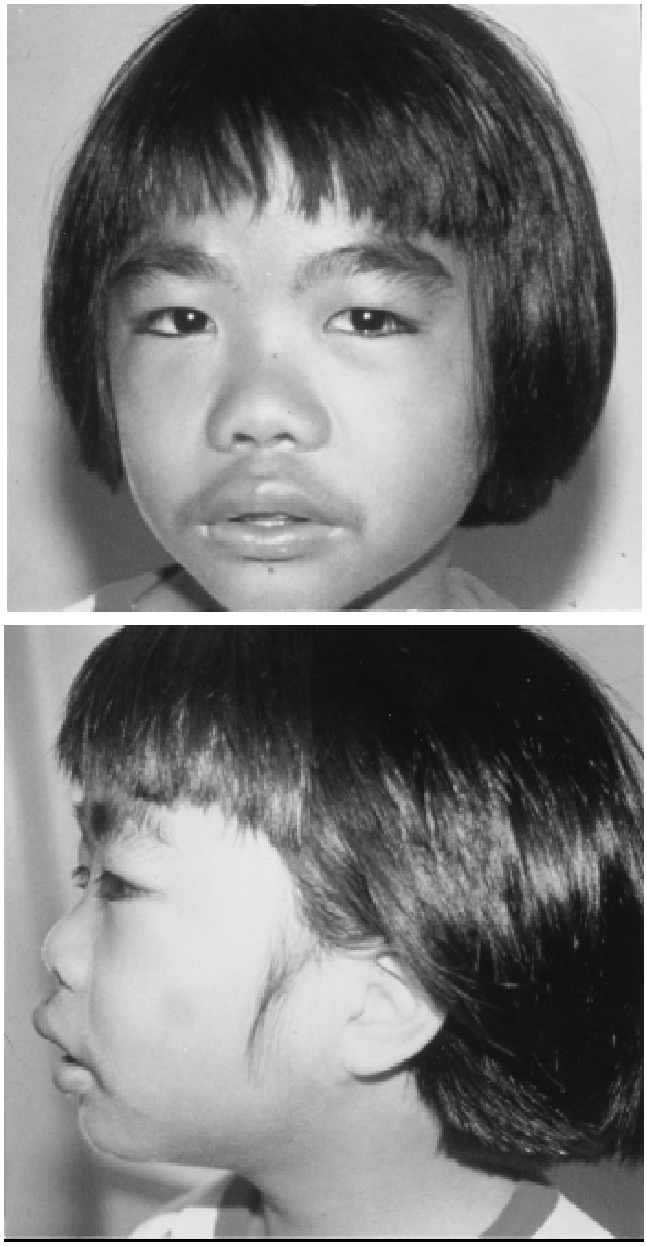

Fig. 1. Frontal and side views of the propositus at age 9 5/12 showing long palpebral fissures, prominent lower lashes, and thick eyebrows as seen in the Kabuki syndrome.

Cytogenetic and FISH Analyses

Metaphase cells from peripheral blood cultures were prepared according to the methods of Yunis [1976]. Cells were studied with standard G-banding techniques as well as with late-replication patterns [Camargo and Červenka, 1984] and fluorescence in situ

hybridization (using instructions provided by the probe manufacturer, Oncor). In situ hybridization was performed with the following probe systems: Y chromosome cocktail (DYZ1 and DYZ3), X chromosome alphoid sequence (DXZ1), X chromosome paint, and XIST.

DNA Polymorphisms and XIST Expression Reference for Two New Markers

DNA samples from the patient and her parents were genotyped with microsatellite markers from the X chromosome: MIC2 [Schmitt et al., 1994], DMD STR-49 [Clemens et al., 1991], DXS1003 [Gyapay et al., 1994], DXS988 [Gyapay et al., 1994], SBMA [Tilley et al., 1989], DXS227 [Fairweather et al., 1993], and DXS101 [Allen and Belmont, 1993]. PCR amplifications were performed with approximately 100 ng of DNA in 25 μl reaction volumes containing: 25 pmol each primer, 10 mM Tris HCl pH 8.3, 50 mM KCl, 1.5 mM $MgCl_2$, 200 μm each dNTP, ≈2 uCi of α32P dCTP (Amersham), and 1.25 U *Taq* polymerase. Samples were subjected to 25–30 cycles of 94 C 1 min, 60–66 C 1 min, and 72 C for 1 min. Amplified PCR products were analyzed on denaturing 5–6% polyacrylamide gels containing 7 M urea and gels were dried and exposed to XAR film (Kodak). The CGG allele sizes at the Fragile-X locus were obtained in a similar fashion, except the PCR reaction was carried out in 15 μl reaction volume using the primer sequences and methods of Fu et al. [1991]. Quantitative Southern blot analysis was performed essentially as described [McGinniss et al., 1992] and sites examined included the Fragile-X locus (probe 5.1/Pst 1 kb fragment) at Xq27.3 [Fu et al., 1991] and the DXYS64 locus (probe St35.239) at Xq28 [Arveiler et al., 1989].

Total RNA was isolated within 30 min from buccal cell scrapings from a normal male, normal female, and the patient using a Micro RNA Isolation kit (Stratagene). Approximately 0.5 μg of RNA was reverse transcribed using an RNA PCR kit (Perkin Elmer) and the resulting cDNA was amplified with primers for the autosomal IL-1α gene (Perkin Elmer), and primers from the XIST gene [Duncan et al., 1993]. PCR products were obtained from cDNA since both primers pairs amplify across an exon/intron boundary. PCR products were isolated on a 2% agarose gel containing size markers (Markers V and VI, Boehringer Mannheim) and detected by ethidium bromide staining.

RESULTS

More than 80% of the patient's blood cells showed a G-banded marker chromosome that was uniformly stained and about one-half the size of the short arm of chromosome 18. Twenty-five of the cells studied with G-banding contained the marker, while another five showed monosomy X. The marker was uniformly dark-staining in all of the 20 marker-containing cells in which late-replication patterns were induced, indicating that these markers had replicated early in the cell cycle.

Further characterization by FISH showed that none of 40 metaphase, or 150 interphase cells, probed with a Y chromosome cocktail exhibited a signal. However, all of the 17 marker-containing metaphases probed with an X-specific alpha satellite probe (DXZ1) exhibited signals over both the X chromosome and the marker (data not shown). Similarly, all of the marker-containing cells studied with the X paint probe exhibited signal over the entire marker (data not shown). One cell probed with DXZ1 showed the marker had a distinct ring morphology with two signals located on opposite sides of the ring. These observations were consistent with this marker being an X-derived ring chromosome. Twenty metaphase cells in which the marker could be clearly visualized had an XIST signal over the normal X but none over the marker (data not shown).

To further characterize the nature and origin of this small-ring X chromosome, we determined the genotypes of the patient and her parents for several microsatellite markers, and used quantitative Southern blotting to assess the genomic copy number for DNA sequences at other loci. Results of these molecular genetic studies indicate this patient was heterozygous at proximal X-linked loci such as SBMA and DXS227 (Table I). Hemizygosity (by PCR and Southern blotting) was observed in DNA samples of the patient at several loci, such as DXS988, DXS1003, and DMD on the short arm, and DXS101, FMR-1, and DXYS64 on the long arm (Table I). These data are consistent with deletion of most of the short arm and much of the distal

TABLE I. Genotyping Results of the Female Patient and Her Parents*

Locus	Probe	Location	Genotype			Origin of r(X)
			Patient	Mother	Father	
MIC2	PCR	Xp22.32; Yp11.3	02 or 22	12	12	—
DMD STR-49	PCR	Xp21.2	02	23	01	Paternal
DXS1003	PCR	Xp11.23–30	02	12	03	Paternal
DXS988	PCR	Xp11.21–22	02	23	01	Paternal
SBMA	PCR	Xq11–q12	12	23	01	—
DXS227	PCR	Xq13.1	12	23	01	—
DXS101	PCR	Xq22	01	13	02	Paternal
FMR-1	5.1/Pst1	Xq27.3	0.43	0.96	0.49	—
FMR-1	PCR	Xq27.3	30	30/30	29	Paternal
DXYS64	St35.239	Xq28	0.53	1.23	1.24	—

*The X chromosome markers are listed according to their map location. The numbers refer to the different alleles. The number of CGG repeats is listed for the PCR analysis at the FMR-1 locus. The densitometric ratios are also listed for each person when analysis was by quantitative Southern blot analysis. The ratio for normal diploid genomic copy number of two should equal ≈ 1.0, ratios should equal ≈ 0.5 for single-copy number.

long arm of the X. Finally, these molecular genetic results indicate this ring X chromosome was paternally derived, since only maternal alleles were inherited by the patient at 5 informative loci: DMD STR-49, DXS1003, DXS988, DXS101, and FMR-1.

Results of RT-PCR experiments on total RNA derived from buccal cells showed no evidence for expression of the XIST gene, since the cDNA samples derived from this patient did not amplify a product of the expected size with the XIST primers (Fig. 2). The cDNA sample from the control female showed evidence for amplification at both the XIST and IL-1α loci. These data complement the results of the FISH analyses, where no XIST signal was detected over the ring X chromosome.

DISCUSSION

The combination of Kabuki syndrome manifestations and a structurally rearranged sex chromosome was noted previously by Niikawa et al. [1988], and Dennis et al. [1993]. We present a female patient with a small-ring X chromosome lacking much of Xp and Xq (including the XIST locus). This patient has the Kabuki syndrome findings of mental retardation, short stature, prominent fingertip pads, and premature adrenarche.

Patients with small-ring X chromosomes typically have more severe congenital anomalies than patients with UTS [Collins et al., 1993; Dennis et al., 1993; Migeon et al., 1993; Jani et al., 1995]. Only recently has the nature of ring X chromosomes been characterized using both molecular and cytogenetic techniques. Jani et al. [1995] examined the genetic content of tiny-ring X chromosomes found in 9 females with severely abnormal phenotypes. The Xp and Xq breakpoints were variable, and three of the nine ring chromosomes were shown to lack the XIST locus. In four rings, the XIST locus was present but not expressed. In the two remaining patients, two ring chromosomes were present—one XIST positive and one XIST negative. The severe phenotype in these two patients was attributed to absence of XIST and inappropriate gene expression from each of the smaller XIST-negative ring chromosomes.

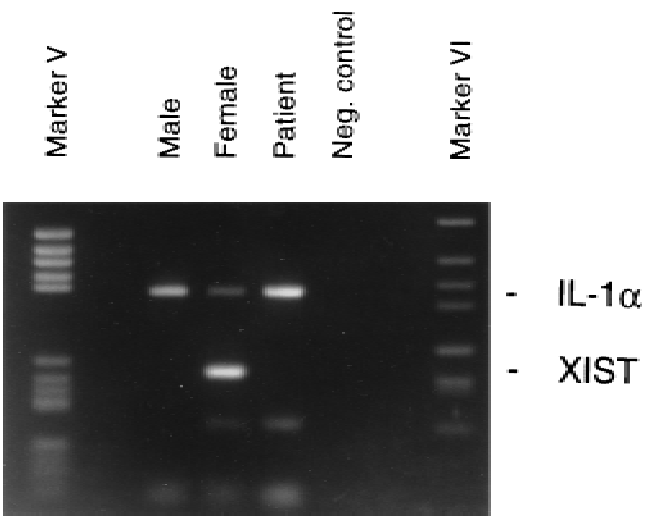

Fig. 2. RT-PCR experiments indicate the XIST gene is not expressed in this patient. cDNA was reverse transcribed from total RNA isolated from buccal cells of the patient and PCR amplified with primers for the IL-1α and the XIST genes. IL-1α is expressed in each sample containing cDNA, but XIST is only expressed in the normal female control and not from the normal male or the patient. Both primer pairs amplify across an exon/intron boundary and therefore do not amplify from DNA.

The clinical findings in these 9 patients with a well-characterized ring X chromosome from Jani et al. [1995] and the present case are summarized in Table II. The molecular genetic data were from Jani et al. [1995] and the clinical descriptions were from the original case reports [Kushnick et al., 1987; Lindgren et al., 1992; Van Dyke et al., 1992; Dennis et al., 1993; Cantu et al., 1995]. Photographs were available on each patient except for patient BT of Jani et al. [1995] (= patient 2 of Lindgren et al. [1992]). Developmental delay was the only abnormality shared by all 10 patients. Syndactyly of fingers or toes (including soft tissue syndactyly) was the most common shared defect seen in 5 of 10 patients. Finally, 4 of 10 patients had neonatal hypotonia, microcephaly, broad nasal bridge, and epicanthus.

Clearly, most patients with an r(X) chromosome and developmental delay do not have many Kabuki syndrome anomalies. Beside the present case, patient AL [Dennis et al., 1993] is the only other patient listed in Table II with several clinical traits of Kabuki syndrome, including long palpebral fissures, arched eyebrows, broad nasal bridge, and short stature. The rings of both these patients are known to contain some of Xp, a centromere, and the AR and DXS227 loci from Xq. Our patient lacks XIST at Xq13.2 and the ring from patient AL was reported to contain the XIST locus although XIST was not expressed. Although similar in size, these two small-ring X chromosomes differ in genetic content.

The genetic basis, if any, of Kabuki syndrome is unknown but the observation of Kabuki-like anomalies in some patients with ring X chromosomes suggests a role for one or more loci on the X chromosome. Some have postulated that microdeletion or haploinsufficiency of genes in the pseudoautosomal region(s) might be responsible for Kabuki syndrome [Niikawa et al., 1988; Hughes and Davies 1994]. However, to account for the equal number of males with Kabuki syndrome there would have to be homologous loci on the Y. One would also expect some males (or females) to have de novo rings or marker chromosomes derived from the Y. Since such patients have yet to be described, it seems unlikely that simple deletion or haploinsufficiency of pseudoautosomal genes is responsible for Kabuki syndrome.

Modulation of gene expression is another mechanism to consider. It is clear that functional disomy of the pericentromeric region of the X chromosome is associated with a severely abnormal phenotype, including congenital anomalies. Whether overexpression of one or more of these genes in this region might be associated with at least some of the manifestations of Kabuki syndrome remains to be seen. However, this mechanism fails to explain the association of Kabuki syndrome and abnormalities of chromosomes other than

TABLE II. Clinical Characteristics of 10 Patients With Well-Characterized Ring X Chromosomes*

	Kushnick et al. [1987]		Lindgren et al. [1992]	Van Dyke et al. [1992]				Dennis et al. [1993]	Cantu et al. [1995]	Present study
Clinical findings	DM	SB	BT	SV (#2)	DC (#3)	TT (#4)	AE (#6)	AL	BP	QL
Face										
Epicanthus				+		+	+			+
Long palpebral fissures								+		+
Everted lateral lower lid										+
Arched eyebrows								+		+
Broad nasal bridge			+	+				+		+
Depressed nasal tip										+
Prominent ears		+							+	–
Other		1	1,2	3		2,4,5	5,6,7	4,8	9,10	
Skeletal abnormalities										
Short fifth finger	–									–
Short fourth metacarpal	–				+	+			+	–
Syndactyly of fingers or toes	+	+		+	+			+		–
Other[a]						Club feet		Joint laxity Thick tapering fingers	Toe anomalies	Short fourth finger
Skin										
Excessive nuchal skin	+		+							–
Fingertip pads	–									+
Dermatoglyphic anomalies	+	+								+
Edematous hands and feet	+		+	+			Lymphe-dema			+
Development										
Developmental delay	+	+	+	+	+	+	+	+	+	+
Neonatal hypotonia	+	+						+		+
Microcephaly	–	+	+	+	–	+	–	–		–
Short stature			+				+	+	+	+
Premature adrenarche										+
Gonadal dysgenesis	–	+								+
Other										
Hearing loss										+
Susceptibility to infections		–			+	+				+
Heart defect			+	+		+				–
Hirsutism		+							+	+
XIST expression	–	–	–	–	–	–/+	–	–	–/+	–

*+, present; –, absent; blank, not documented.
[a]Other facial features: 1, hypertelorism; 2, micrognathia; 3, facial asymmetry; 4, low-set ears; 5, high arched palate; 6, strabismus; 7, telocanthus; 8, thin upper lip; 9, webbed neck; 10, triangular facies.

the X chromosome. Alternatively, one could postulate that mutations of a gene or genes important in regulation of gene transcription might play a role in Kabuki syndrome. For example, mutations in the XH2 gene at Xq13.3 are associated with down regulation of alpha globin synthesis from chromosome 16 in boys affected with X-linked mental retardation with α-thalassemia (ATR-X syndrome) [Gibbons et al., 1995]. Thus, it is possible that mutations in a regulatory gene on any chromosome could specifically modulate gene expression at one or more target genes (pericentromeric region of the X or other regions) and be associated with Kabuki syndrome.

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